IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re-application of
Tanja OUIMET et al.

Group art Unit : 1652

Serial N° : 09/647,780

Examiner : Malgorzata Walicka A.

Filed : December 26, 2000

For : Novel membrane-bound metalloprotease NEP 2 and the use thereof for screening inhibitors useful in therapy to be used in the treatment of tumours with cytotoxic agents

DECLARATION UNDER RULE 132

Hon. Commissioner of Patents and Trademarks
WASHINGTON D.C. 20231

Sir :

I, Tanja Ouimet, residing at 9, Villa Juge, 75015 Paris
Declare and say :

I am citizen of Canada and Greece

I am PhD graduate from the University of Montreal, Montreal, Canada.

I am currently working at the research laboratory of INSERM Unit 573 (France).

I am an inventor of the present patent application and I am aware that the Examiner alleged that th specification of the application would fail to establish a function and substantial utility for the polypeptide according to the invention.

However, I believe that the additional experimental data provided herein clearly demonstrate that the claimed human polypeptide shows an enzymatic activity characteristic of a metalloprotease, and which parallels that of rat NEP2.

The full length sequence of human NEP2 (hNEP2) comprises the amino acid sequence encoded by the nucleic acid sequence shown in the instant application as SEQ ID No:3. Since hNEP2 is a membrane proteinase, the kinetic parameters of the enzyme were determined by using a soluble form of hNEP2 (hNEP2s) that lacks the transmembrane domain.

I personally conducted experiments to characterize hNEP2s activity towards the model substrates Suc-AAF-AMC (succinyl-Ala-Ala-Phe-7-amido-4-methylcoumarin) and MeOSuc-GLF-AMC (3-methoxysuccinyl-Gly-Leu-Phe-7-amido-4-methylcoumarin). The kinetic parameters of hNEP2s are reported in the table below and compared with those determined for soluble rat NEP2 (rNEP2s), as published in Rose et al. (Biochem J. (2002) 363, 697-705).

	hNEP2s K_M (μM)	rNEP2s K_M (μM)
Suc-AAF-AMC	38	50
MeOSuc-GLF-AMC	48	35

The cleavage site of Suc-AAF-AMC and MeOSuc-GLF-AMC was found to be same for both hNEP2s and rNEP2s, i.e. Suc-AA---F-AMC and MeOSuc-G---LF-AMC, respectively.

The enzymatic activity of hNEP2s was further characterized by determining the capacity of hNEP2s to hydrolyze two natural peptides by

assessing the inhibitory potency of these natural peptides towards Suc-AAF-AMC cleavage by hNEP2s:

	Ki (μM)	
	hNEP2s	rNEP2s
gonadotropin-releasing hormone (GnRH)	5.4	18
Met-enkephalin	9	10

At last the synthetic substrate Suc-AAF-AMC was used to evaluate the inhibitory potency of three metalloprotease inhibitors

	Ki (nM)	
	hNEP2s	rNEP2s
Phosphoramidon	2.2	0.8
Thiorphan	483	84
Omapatrilat	12	4.4

Altogether, the results provided herein demonstrate that the enzyme identified as human NEP2 shows proteolytic activity towards synthetic and natural substrates known to be hydrolyzed by neprilysin, the prototypal member of a metalloprotease subfamily. Human NEP2 activity was further found to be inhibited by inhibitors characteristic of metalloproteases.

Moreover, these results show that human and rat NEP2 display comparable proteolytic activity and inhibitory pattern.

Accordingly, the claimed human polypeptide shows an enzymatic activity characteristic of a metalloprotease that parallels that of rat NEP2.

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The undersigned Declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 7th day of november 2003

Tanja Quinet